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Furan

MAK Value Documentation, supplement – Translation of the German version from 2017

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Keywords

furan, cholangiocarcinoma, low-dose carcinogenicity, hepatotoxicity, bile-duct cells, inflammation, maximum workplace concentration, MAK value, skin absorption

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated furan [110-00-9]. All toxicity endpoints have been considered and publications are described in detail.

Genotoxicity data show only a secondary genotoxic effect at high doses and no effect at low doses. Therefore, furan is classified as a primary non-genotoxic carcinogen. As a maximum concentration at the workplace (MAK value) can be derived from available studies, furan was reclassified from Category 2 into Category 4 for carcinogens.

There are no long-term inhalation studies available to derive a MAK value. In a 13-week study with rats, subcapsular inflammation and apoptosis in the liver of unclear relevance for humans were observed at 0.12 mg/kg body weight/day with a NOAEL of 0.03 mg/kg body weight/day. These may be adaptive effects and were not observed in a two-year study or its interim investigations. A physiologically based pharmacokinetic model predicted the burden in human livers to be about three-fold lower compared with rats. The NOAEL of 0.03 mg/kg body weight/day was scaled to a concentration at the workplace and a MAK value of 0.02 ml/m³ was derived, also taking into account the lower liver dose in humans.

The MAK value was derived from a systemic NOAEL; therefore, furan is classified in Peak Limitation Category II with the default excursion factor of 2 as sufficient toxicokinetic data are not available, especially for the reactive metabolite cis-2-butene-1,4-dial.

Model calculations show that dermal absorption would contribute substantially to the systemic toxicity and furan continues to be designated with an “H”.

Because there are no studies on developmental toxicity, furan is assigned to Pregnancy Risk Group D.

There are no data on sensitization.

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MAK value (2016)	**0.02 ml/m³ ≙ 0.056 mg/m³**
Peak limitation (2016)	**Category II, excursion factor 2**
Absorption through the skin (2005)	**H**
Sensitization	**–**
Carcinogenicity (2016)	**Category 4**
Prenatal toxicity (2016)	**Pregnancy Risk Group D**
Germ cell mutagenicity	**–**
BAT value	**–**
Molar mass	68.08 g/mol
Melting point	−85.6 °C (ECHA 2015)
Boiling point at 1013 hPa	31.5 °C (ECHA 2015)
Density at 20 °C	0.95 g/cm³ (ECHA 2015)
Vapour pressure at 25 °C	798 or 860 hPa (ECHA 2015)
log K_{OW}	1.34 (ECHA 2015)
Solubility at 25 °C	10 g/l water (ECHA 2015)
1 ml/m³ (ppm) ≙ 2.825 mg/m³	**1 mg/m³ ≙ 0.354 ml/m³ (ppm)**

Documentation for furan was published in 2006 (english translation Hartwig and MAK Commission 2018). Furan was designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts) and classified in Carcinogen Category 2 after two-year oral studies found that furan induced liver adenomas and carcinomas in rats and mice and bile duct carcinomas in rats at the lowest dose tested and above. In the meantime, studies have become available that established doses without effects. Therefore, the studies have been reviewed to derive a MAK value.

Furan can be found in heat-processed foods, for example preserved meat and vegetables, vegetable juices, soups, bread and brewed coffee. The WHO reported that Europeans have an average daily intake of 0.27–1.17 µg/kg body weight, but the intake may be as high as 2.2 µg/kg body weight if, for example, coffee is consumed in large quantities (Churchwell et al. 2015).

1 Toxic Effects and Mode of Action

Data published since the 2006 documentation (Hartwig and MAK Commission 2018) confirmed the previously described mode of action for furan. In rats and mice, oral doses of furan induced carcinogenicity primarily in the liver and secondary genotoxicity occurred only at cytotoxic concentrations. Apoptosis of hepatocytes was observed when gavage doses of 0.12 mg/kg body weight and day and above were administered to F344 rats for 13 weeks. A two-year study with male F344 rats reported cholangiofibrosis in 76% of the animals at gavage doses of 0.2 mg/kg body weight and day and above; 1 of 100 animals was affected at the dose of 0.092 mg/kg body

weight and day. When mice were gavaged with furan for 13 weeks, lesions in the liver lobules were observed at 0.5 mg/kg body weight and day and above. Furan is metabolized in the liver mainly by cytochrome P450 (CYP) 2E1. *cis*-2-Butene-1,4-dial is formed as a reactive metabolite and contributes to the hepatotoxicity only after exposure to furan at high doses. There are no inhalation studies available.

2 Mechanism of Action

The 2006 documentation (Hartwig and MAK Commission 2018) considered the tumour-promoting activity of furan to be the main cause of its carcinogenicity. This is induced by cytotoxicity, primarily by the uncoupling of oxidative phosphorylation (mitochondrial toxicity) and the subsequent reduced formation of adenosine triphosphate (ATP). The cytotoxicity is probably caused by the metabolite *cis*-2-butene-1,4-dial and leads to apoptosis. Unspecific reactions cause necrosis at higher doses. Hepatocytes can survive a decrease in ATP of up to 95%; therefore, the misrepair of DNA breaks or the manifestation of lesions as a result of increased proliferation and the conversion of liver tissue is assumed to be an additional effect.

Since the 2006 documentation new studies have been carried out that are described in detail below (see also Table 1).

2.1 Hepatotoxicity

Epigenetic changes in the liver that may have impaired the cell cycle control were observed at 4.4 mg/kg body weight and day and above in F344 rats given gavage doses of furan for 26 weeks and at 0.92 mg/kg body weight and day and above in rats exposed for 1 year (see Table 1). The carcinogenicity was attributed to a non-genotoxic mechanism in the form of cytotoxicity, inflammation, oxidative stress, demethylation of the liver DNA and increased cell proliferation (De Conti et al. 2014).

Rats were administered gavage doses of furan of up to 30 mg/kg body weight and day for periods of 1 day to 3 months to investigate the time-dependent changes in the liver (see Table 1). Depending on the extent of the damage around the central vein of the liver lobules, a rapid proliferation of hepatocytes was observed, or the proliferation of ductular cells when the damage extended into the periportal field. The ductular cells extended into the parenchyma and were accompanied by liver fibroblasts (Hickling et al. 2010 a). They either differentiated into hepatocytes, with the loss of the associated fibroblasts, or progressed to form tortuous ductular structures that replaced the parenchyma and led to cholangiofibrosis. The latter was observed mainly in regions of the liver lobules that had been completely destroyed at the beginning of treatment. The mechanism that determined the direction of differentiation was unclear. It was assumed to be irreversible disturbance of the repair system of the hepatocellular stem cells (Hickling et al. 2010 a, b). As the CYP2E1 expression was considerably reduced in the regenerative hepatocytes at constant levels of furan exposure, these were clearly more resistant, resulting in reduced compensatory proliferation. However, inflammation was found to persist at the affected sites of the liver lobes even after termination of the furan treatment; this induced oxidative stress in the surrounding tissue. The carcinogenicity of furan was considered to be the result of a combination of effects including cytotoxicity, persistent indirect damage to the DNA as a result of oxygen radicals, conversion of the DNA damage to fixed mutations by persistent proliferation and changes in the gene expression associated with cell cycle control (Hickling et al. 2010 b).

A study in female B6C3F1 mice that were given oral doses of furan for 3 weeks or 2 years showed that tumours were not caused by hepatic cytotoxicity alone. The doses of 1 and 2 mg/kg body weight and day induced hepatic cytotoxicity but did not lead to tumour formation. Hepatotoxicity and compensatory cell proliferation were induced at doses of 4 and 8 mg/kg body weight and day, which led to foci of altered hepatocytes, hepatocellular adenomas and, at 8 mg/kg body weight and day, to hepatocellular carcinomas (Moser et al. 2009).

Mechanistic studies in rats that were administered gavage doses of furan for up to 3 months reported changes in the gene expression patterns at lower doses over time (Cooke et al. 2014; Hickling et al. 2010 b).

In hepatocellular carcinomas of humans and rodents, the expression of the tumour suppressor genes *Cdh1, p16^{INK4}* and *Rassf1a* was frequently down-regulated by methylation. A study with oral furan doses of up to 4.4 mg/kg body weight and day that were administered to rats for periods of 13, 26 or 53 weeks reported time and dose-dependent methylation of *p16^{INK4}* and *Rassf1a* (De Conti et al. 2014).

Tab. 1 Mechanistic studies of hepatotoxicity after repeated oral administration of furan

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 5 ♂	**1 day**, **1 month**, **3 months**, **3 months with a 1-month recovery period**, 0, 30 mg/kg body weight and day, 5 days/week, in corn oil, purity: > 99%, **gavage**	**examination of liver only**, examination of all liver lobes, immunocytochemistry with 3 animals/group, control animals after 1 day, 1 month, 3 months, **pathology** day 7: liver pale with swelling and distortion of the caudate and right anterior liver lobes, increasing up to days 10 and 12, additionally nodules, on day 20: fusion of the 2 liver lobes in some cases, after 3 months: all liver lobes pale, swollen with nodules and changes in structure, **histopathology/immunocytology** 8 hours: in centrilobular and subcapsular regions of all liver lobules: vacuolation, chromatin aggregation, karyorrhexis, karyolysis → necrosis and apoptosis, 24 hours: influx of inflammatory cells in the case of subcapsular and centrilobular necrosis, 3 days: most necrosis, inflammation, fibronectin deposits in centrilobular region resolved, centrilobular parenchyma is replaced by hepatocytes formed by proliferation, surviving hepatocytes proliferate, portal regions previously largely destroyed: proliferation of OV-6-positive ductular hepatocytes, 7 days: proliferating ductular hepatocytes extending into the parenchyma, 12 days: new ductular hepatocytes show typical and atypical differentiations, typical differentiation in portal regions in continuity with hepatic plates, distinct boundary between cells without bile duct markers OV-6 and BD1 and Cx43, atypical differentiations into intestinal cells in regions that had largely been destroyed, 20 days: proliferating ductular hepatocytes replace most of the parenchyma in destroyed areas of the right and caudate lobes of the liver, > 20 days to 3 months (including 3 months with 1-month recovery period): sites of cholangiofibrosis expand accompanied by chronic inflammatory infiltrates, density of Cx32 spots on remaining intact hepatocyte islands decrease and no longer increase even in exposure-free periods	Hickling et al. 2010 a
rat, Sprague Dawley, 5 ♂	**1 day**, **1 month**, **3 months**, **3 months with a 1-month recovery period**, 0, 30 mg/kg body weight and day, 5 days/week, in corn oil, purity: > 99%, **gavage**	**examination of liver only**, immunohistochemistry with 3 animals/group, histopathology: see Hickling et al. 2010 a, CYP2E1: induced following acute toxicity, normal on day 3 and thereafter, marker for cell proliferation and DNA damage: wild type p53, cdk-1 (for mitosis), mdm-2, phospho-c-jun apparent in surviving hepatocytes after acute exposure, 8-oxo-dG ↑ in hepatocytes and ductular hepatocytes, changes in gene expression after 3-month exposure and 1-month recovery period: *HSP 70, GST, IRF-1*, cytochrome b558 for cellular/oxidative stress; for example *cyclin E, annexin II, c-myc* for cell proliferation; *TB-10, mANT* for apoptosis; *carbonic anhydrase II, TB-10* for tumour progression; cutaneous fatty acid-binding protein S-100 for metastases	Hickling et al. 2010 b

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, F344, 12 ♂	**13 weeks**, 0, 0.03, 0.12, 0.5, 2.0, 8.0 mg/kg body weight and day, 5 days/week, purity: no data, **gavage**	**scope of examinations**: body weights, organ weights, histopathology of prostate gland, testes, epididymis, seminal vesicles, pituitary gland; serum and intratesticular testosterone levels; serum LH and FSH levels; 17-OHase, 3-beta-HSD and 17-beta-HSD activities, mRNA expression levels of the LH receptor and Tspo, **0.03 mg/kg body weight and above**: StAR ↑; **2 mg/kg body weight and above**: serum testosterone level ↑, serum LH ↓; **8 mg/kg body weight**: intratesticular testosterone level ↑, 25% decrease in serum LH, cholesterol side-chain cleavage enzyme (Cyp11a1) ↑	Cooke et al. 2014
rat, F344/N, 5 ♂, 5 ♀	**13 weeks**, **26 weeks**, **1 year**, 0, 0.92, 2.0, 4.4 mg/kg body weight and day, 5 days/week, in corn oil, purity: no data, **gavage**	**examination of liver only** **scope of examinations**: change in the status of global DNA methylation, promoter methylation, expression of tumour suppressor genes, expression of chromatin-modifying genes, **13 weeks**: **4.4 mg/kg body weight**: *Dnmt3b* ↓, **26 weeks**: trimethylated histones dose-dependently ↓ (H3K9 and H4K20), **4.4 mg/kg body weight**: 4/5 animals: methylated $p16^{INK4}$ ↓, 5/6 animals: methylated *Rassf1a, Dnmt3b* ↓, **1 year**: trimethylated histones dose-dependently ↓ (H3K9 35%, H4K20 36%), histone acetyltransferases dose-dependently ↓ (*Ep300* and *Kat2a*), **0.92 mg/kg body weight**: DNA methylation 30%, 36% and 49% ↓, dose-dependent hypermethylation of tumour suppressor gene $p16^{INK4a}$; **2.0 mg/kg body weight**: dose-dependent hypermethylation of tumour suppressor gene *Rassf1a*; **4.4 mg/kg body weight**: *Dnmt3a* ↓, *Dnmt3b* ↓, H3K9ac 45% ↓, H3K56ac 32% ↓, histone-modifying genes *Prdm2* and *Suv39h1* and *Suv4-20h2* and histone methyltransferases *Ehmt2* ↓, no changes in *Cdh1, Dnmt1,* trimethylated histones H3K4, H3K27	de Conti et al. 2014

BrdU: 5-bromo-2′-deoxyuridine; Cx32: connexin 32; FSH: follicle-stimulating hormone; 3-beta-HSD: 3-beta-hydroxysteroid dehydrogenase; 17-beta-HSD: 17-beta-hydroxysteroid dehydrogenase; LH: luteinizing hormone; LI: labelling index; 17-OHase: 17-hydroxylase, C17,20-lyase; OV-6: specific antibody for the identification of hepatic or ductular precursor cells; SPF: specific pathogen free; StAR: steroidogenic acute regulatory protein; Tspo: translocator protein

2.2 Genotoxicity

Recent studies likewise showed that the carcinogenicity of furan was not caused by primary genotoxic effects in vivo (Carthew et al. 2010; Chen et al. 2010; Ding et al. 2012; Durling et al. 2007). As *cis*-2-butene-1,4-dial did not bind to DNA in vivo (rats), furan is not thought to have a genotoxic mechanism of action (Churchwell et al. 2015). However, some studies reported (reversible) changes in gene expression governing cell cycle control and apoptosis that were caused by furan after oral treatment of rats for 4 or 13 weeks (Chen et al. 2010, 2012). In mice, an increase in polyploid cells and DNA repair genes was observed at the highest dose tested of 15 mg/kg body weight and day (Cordelli et al. 2010). Oxidative stress, which was accompanied by inflammation, cell proliferation and cytotoxicity, was regarded as having caused the effects (Ding et al. 2012); they were not associated with a change in DNA methylation (Chen et al. 2010; Cordelli et al. 2010). The genes that were activated among those that play an important role in cytotoxicity and proliferation were *stress-activated protein kinase (SAPK)* and *death receptor (DR5, TNF-alpha), extracellular signal-regulated kinases (ERKs)* and *TNF-alpha* and the genes *NF-kappaB* and *c-Jun*, which are relevant for liver regeneration. *NRF2*, the master gene for oxidative stress and chronic inflammation, was activated in particular (Jackson et al. 2014). The mitochondria are regarded as a critical target; their energy supply was impaired by the irreversible uncoupling of oxidative phosphorylation caused by furan and ATP depletion (WHO 2011). Some proteins lost their function as a result of covalent bindings and misfolding (Moro et al. 2012).

2.3 Summary

An individual mechanism that could be responsible for tumourigenicity cannot be determined from the data. Furan exhibited a high level of cytotoxicity. This is presumably caused by *cis*-2-butene-1,4-dial, which is formed by CYP2E1 during metabolism. *cis*-2-Butene-1,4-dial is a very reactive substance that binds to glutathione (GSH), amino acids or DNA in vitro, but has not been demonstrated in vivo, and can lead to DNA cross-links. Furan induces changes in gene expression, some of them irreversible, as well as cytotoxicity, oxidative stress and proliferation. Low oral doses of furan did not cause genotoxic effects, whereas secondary genotoxicity was observed after administration of high doses. In addition, uncoupling of oxidative phosphorylation and ATP depletion in the mitochondria were reported. Furan induces CYP2E1 and hepatocellular damage around the central vein, which is followed by apoptosis. If the damage is slight, tissue regeneration originates from the hepatocytes; this tissue is characterized by a much lower level of CYP2E1 expression resulting in the formation of a smaller amount of *cis*-2-butene-1,4-dial. If the areas damaged by high doses of furan extend into the periportal field, which contains ductular cells, hepatocytes and severely proliferating ductular cells cause tissue regeneration. Intestinal cells may also be formed in the liver lobule. Medium-term or long-term administration of furan induces continuous tissue proliferation, which in turn causes cholangiofibrosis resulting in cholangiocarcinomas and hepatocellular carcinomas.

Inhalation studies and thus investigations of local toxicity in the respiratory tract were not carried out.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

The retention of inhaled furan in dogs was greater than 90% (Hartwig and MAK Commission 2018).

After the administration of single gavage doses of ^{14}C-labelled furan, 14% of the radioactivity was exhaled as unchanged furan and 26% as CO_2, 20% of the radioactivity was found in the urine and another 22% was eliminated in the faeces within the first 24 hours. After 24 hours, 68% of the 19% that remained in the body (13% of the administered dose) was detected in the liver, bound to macromolecules (Hartwig and MAK Commission 2018).

Fischer 344 rats were gavaged with a furan dose of 0.92 mg/kg body weight and blood samples were taken after 15, 30, 45, 60 and 90 minutes and after 2, 3, 4, 5 and 6 hours to investigate the toxicokinetics of the substance in serum and liver. The maximum furan concentration in the blood of 63 ± 17 nM was obtained within the first 15 minutes following administration; the concentration then decreased with a half-life of 1.3 hours. The maximum furan concentration in the liver of 547 ± 993 pmol/g tissue was determined 30 minutes after administration of the substance. It was highly variable and dependent on the time that had passed since administration and the localization of the liver lobule that was investigated. The relative standard deviation of the furan concentration between the liver lobules was 65%; the same liver lobule was examined from each of the 4 animals. There was a biphasic decrease in the concentration in the liver with half-lives of 0.55 and 0.62 hours. Furan was no longer detected in the liver or blood 8 hours after administration. The area under the concentration-time curve that was extrapolated to infinity was 772 pmol/g × hour for the liver and 139 nmol/l × hour for the blood. At all sampling times, the furan concentration in the liver was greater than that in the blood with a mean ratio of about 6.2 (Churchwell et al. 2015).

As the oral route of administration was used in this study, furan first entered the liver and then the systemic blood. The liver was thus exposed to a higher concentration of furan than the blood. This does not conflict with the assessment that after inhalation the concentration in the blood is about 10 times as high as that in the liver and the blood flow in the liver is the rate-limiting step (Churchwell et al. 2015; Kedderis et al. 1993).

Fluxes of 759, 62 and 101 µg/cm^2 and hour were calculated for a saturated aqueous solution using the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995), respectively. Assuming the

exposure of a 2000 cm^2 surface area of skin for 1 hour, this would correspond to absorbed amounts of 1518, 124 and 202 mg, respectively.

3.2 Metabolism

3.2.1 Humans

Oxidation by CYP2E1 to form *cis*-2-butene-1,4-dial, which binds primarily to thiols, amino groups or amino acids, is the first step in the metabolism of furan. In vitro studies demonstrated a similar metabolic pathway for humans, rats and mice. According to a physiologically based pharmacokinetic (PBPK) model, the burden per kg body weight caused by furan and the exposure to the reactive metabolite *cis*-2-butene-1,4-dial in the liver was 3 and 10 times lower in humans than that in rats and mice, respectively, after exposure to the same external furan concentration of 10 ml/m^3 (Hartwig and MAK Commission 2018; Kedderis and Held 1996).

3.2.2 Rats

Furan is oxidized by CYP to form *cis*-2-butene-1,4-dial. Studies found the following metabolites in the urine of rats treated with furan: a monoglutathione-butenedial reaction product, *N*-[4-carboxy-4-(3-mercapto-1*H*-pyrrol-1-yl)-1-oxobutyl]-L-cysteinylglycine cyclic sulfide, *R*-2-acetylamino-6-(2,5-dihydro-2-oxo-1*H*-pyrrol-1-yl)-1-hexanoic acid, *N*-acetyl-*S*-[1-(5-acetylamino-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine and its sulfoxide. To investigate the quantitative course of metabolism, the metabolites were quantified in the 24-hour urine of male F344 rats after single gavage doses of ring-labelled furan of 8 or 40 mg/kg body weight. As only trace amounts of *N*-acetyl-*S*-[1-(5-acetylamino-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine ("end product" of the reaction of 2-butene-1,4-dial with GSH) was detected in the urine, two main in vivo pathways were identified: the subsequent binding to nucleophilic macromolecules or the direct binding of 2-butene-1,4-dial to macromolecules (Lu et al. 2009).

Studies with rat hepatocytes showed that the metabolite *cis*-2-butene-1,4-dial reacted with glutathione to form 2-(*S*-glutathionyl)succinaldehyde, which forms pyrrole cross-links with cellular amines such as lysine and glutamine and also with ornithine, putrescine and spermidine. Mercapturic acid derivatives of the spermidine cross-links were detected in the urine of rats treated with furan. The authors concluded that this metabolic pathway occurs in vivo (Peterson et al. 2011).

A single [3,4-^{13}C] furan dose of 5 mg/kg body weight was administered to 3 bile duct-cannulated rats and the metabolites were recovered in the bile in 30-minute fractions over a period of 4 hours and analysed. The cyclic monoglutathione conjugate of *cis*-2-butene-1,4-dial, an *N*-acetylcysteine-*N*-acetyllysine conjugate and a cysteinylglycine-glutathione conjugate were identified as the main metabolites. All these metabolites had previously been detected in the urine of exposed rats (Hamberger et al. 2010).

4 Effects in Humans

There are no data available.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

There are no new data available.

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

There are no new data available. Earlier studies that were included in the 2006 documentation (Hartwig and MAK Commission 2018) were documented inadequately and have not been included in the evaluation.

5.2.2 Oral administration

The liver is the main target organ of furan. A 2-year study with rats and mice reported a dose-dependent increase in tumours in the liver at the low dose and above (see Section 5.7; Hartwig and MAK Commission 2018).

Studies with repeated oral administration of furan that were not included in the 2006 documentation (Hartwig and MAK Commission 2018) are shown in Table 2 to 6.

Initially, low gavage doses of furan induced only hepatotoxicity in rats and mice. A NOAEL (no observed adverse effect level) of 0.03 mg/kg body weight and day was determined in F344 rats after 13-week administration; at 0.12 mg/kg body weight and day and above, slight histopathological changes were observed in the caudate lobe of the liver that increased in severity with the dose. In addition, apoptosis of hepatocytes, Kupffer cells with yellow pigment deposits and foci of subcapsular inflammatory cells were found. Up to 8 mg/kg body weight and day, substance-induced findings were not observed in any other organ, whereas the findings in the liver increased in a dose-dependent manner. The first effects may appear in the caudate lobe because the caudate lobe is located closest to the stomach and therefore diffusion into liver tissue may have taken place. The animals for the study were obtained from Charles River, Quebec (see Tables 2 and 3; Gill et al. 2010).

A NOAEL of 0.092 mg/kg body weight and day was determined by a study with two-year gavage administration of furan to male F344 rats; at 0.2 mg/kg body weight and above, cholangiofibrosis was observed in 76% of the animals (see Table 6). The interim examinations after 9 and 15 months showed that the NOAEL decreased with an increase in time (see Tables 4 and 5). The hepatotoxicity increased in a dose-dependent manner up to the highest dose tested of 2 mg/kg body weight and day; cholangiocarcinomas were not observed (FDA 2015). Meanwhile the study by FDA (2015) has been published (Von Tungeln et al. 2017).

It is unclear why the sensitivity of hepatocytes and bile duct cells differed in the 13-week and two-year studies. Slight genetic differences may have been responsible because the animals of the 13-week study were obtained from Charles River in Quebec, Canada, and the animals of the two-year study were bred by the National Center for Toxicological Research in the United States.

Gavage studies that were carried out for 3 weeks, 13 weeks and 2 years are available for mice; they confirmed that the liver is the most sensitive target organ. After 13-week administration of furan, lesions in the liver lobule were observed at 0.5 mg/kg body weight and day and above; the NOAEL was 0.12 mg/kg body weight and day. In the two-year study, histopathological findings were observed only in the liver; subcapsular inflammation was found at the low dose of 0.5 mg/kg body weight and day (see also section 5.7; Gill et al. 2011; Moser et al. 2009).

Tab. 2 Studies of the toxicity of furan after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, Fischer 344/N, 12 ♂, 12 ♀	**13 weeks**, 0, 0.03, 0.12, 0.5, 2.0, 8.0 mg/kg body weight and day, 5 days/week, in corn oil, purity: > 99%, **gavage**	**0.03 mg/kg body weight**: **NOAEL for hepatotoxicity**; **0.12 mg/kg body weight and above**: changes in the caudate lobe of the liver (see Table 3), hepatocyte apoptosis (♂), Kupffer cells with yellow pigment deposits (♂), foci of subcapsular inflammatory cells; biochemical parameters: T4 (♂) ↑; **0.5 mg/kg body weight and above**: in all animals, changes in the caudate lobe of the liver, similar changes in the left liver lobe; cell layer thickness increased with hepatocytes with cytomegaly and karyomegaly and dark basophilic cytoplasm, hepatocyte apoptosis, Kupffer cells with yellow pigment deposits ↑ (♀); biochemical parameters: triglycerides (♂) ↓, number of platelets (♀) ↑; **2.0 mg/kg body weight and above**: subcapsular and periportal proliferation of oval cells, bile duct hyperplasia, infiltrates: inflammatory cells, fibrosis; biochemical parameters: ALT (♀) ↓, ALP (♂) ↑, albumin (♀) ↑, amylase (♂) ↓, globulin (♂) ↓, glucose ↓, total protein (♀) ↑, triglycerides (♀) ↓, phosphorus (♀) ↑, T3 (♀) ↑; **8.0 mg/kg body weight**: 14%–22% increase in absolute liver weights, nodules in liver (mainly in the caudate and left lateral liver lobes), histopathological changes in all liver lobes, biliary epithelial and oval cell hyperplasia, cholangiofibrosis instead of hepatic parenchyma; biochemical parameters: ALT ↓, albumin ↑, cholesterol (♂) ↑, number of platelets ↑, %$CD4^+CD8^+$ (♂) ↓, %$CD4^+CD8^-$ (♂) ↑ (♀ also trend) (possibly thymus toxicity); scope of examinations: feed consumption, body and organ weights; in all dose groups: histopathology of liver, kidneys, thymus, spleen; only control and highest dose group: histopathology of stomach, thyroid gland, adrenal glands, pancreas, testes, epididymis, prostate gland, seminal vesicles, ovaries, uterus and vagina; only organ weight determined: heart; paired organs weighed separately; end of study: biochemical parameters in serum, blood parameters, thymocyte phenotyping	Gill et al. 2010
rat, F344, 80 (to 190) ♂, 7 weeks old at beginning of study	**2 years**, interim examination after 9 (n = 20) and 15 (n = 10) months, 0 (n = 190), 0.02 (n = 180), 0.044 (n = 130), 0.092 (n = 130), 0.2, 0.44, 0.92, 2 mg/kg body weight and day, 5 days/week, in corn oil, purity: 99.5%, **gavage**	**2 years**, for findings in the liver see Table 4 (9 months), Table 5 (15 months), Table 6 (2 years), **0.092 mg/kg body weight** (n = 100): **NOAEL**; **0.2 mg/kg body weight and above** (n = 50): hepatotoxicity; after 2 years: cholangiofibrosis in 76% of the animals (see Table 6); **2 mg/kg body weight** (n = 50): hyperplasia of myeloid cells in the bone marrow (43%**, controls: 24%), cataract in the eye (13%**, controls: 1%), oedema in the forestomach (31%*, controls: 18%), hyperplasia in the epithelium of the forestomach (44%*, controls: 28%), chronic inflammation of the forestomach (40%*, controls: 22%), ulcer in the forestomach (19%*, controls: 9%), hyperplasia in the transitional epithelium of the kidneys (38%**, controls: 18%), sinus dilation in the pancreatic lymph node (12%**, controls: 0%), hyperplasia in the parathyroid gland (38%*, controls: 26%), cysts on the skin (12%**, controls: 3%), follicular cysts in the thyroid gland (14%*, controls: 5%), mineralization in testis (6%*, controls: 1%)	FDA 2015

Tab. 2 *(continued)*

Species, strain, number per group	Exposure	Findings	References
mouse, SPF B6C3F1, 15 ♀, only ♀ animals because of lower spontaneous liver tumour incidence (14%, ♂: 52%) and because both sexes generally react in the same way	**3 weeks**, 0, 0.5, 1, 2, 4, 8 mg/kg body weight and day, 5 days/week, in corn oil, purity: > 99%, **gavage**	end of study: separate and histopathological examination of liver lobes only; **0.5 mg/kg body weight and above**: hepatotoxicity (degeneration of hepatic parenchyma, inflammation or subcapsular necrosis and inflammation in hepatocytes on an area with contact with forestomach), trend towards increased ALT, trend towards increased cell proliferation (BrdU) and labelling index; **1 mg/kg body weight and above**: ALT ↑, incidence of mitotic figures in hepatocytes ↑, statistically significant hepatotoxicity; **2 mg/kg body weight and above**: positive trend towards increased labelling index; **4 mg/kg body weight and above**: absolute and relative liver weights ↑; **8 mg/kg body weight**: labelling index ↑	Moser et al. 2009
mouse, B6C3F1, 16 ♂, 16 ♀, 5 for histopathology (reported), 11 for molecular and protein analysis (results not reported)	**13 weeks**, 0, 0.03, 0.12, 0.5, 2.0, 8.0 mg/kg body weight and day, 5 days/week, in corn oil, purity: > 99%, **gavage**	**0.12 mg/kg body weight**: **NOAEL for hepatotoxicity**, phosphorus (♂) ↑; **0.5 mg/kg body weight and above**: blood urea ↓, (subcapsular) lesions in the caudate liver lobe; **2 mg/kg body weight and above**: serum amylase (♀) ↓, caudate liver lobes: hepatocyte apoptosis, Kupffer cells with yellow pigment deposits, oval cell hyperplasia, inflammatory infiltrates, border of enlarged hepatocytes with basophilic cytoplasm and nucleoli between parenchyma and subcapsular lesions, left lobe: subcapsular hepatocyte apoptosis and Kupffer cells with pigment deposits, inflammatory infiltrates, right lobe: similar findings in 1 ♂ and 1 ♀; **8 mg/kg body weight**: 14% increase in absolute and relative liver weights (♀), ALT ↑, uric acid (♂) ↑, findings in the right liver lobe in 2 ♂ and 2 ♀, apoptotic hepatocytes in the parenchyma (♀), bile duct hyperplasia; scope of examinations: feed consumption, body and organ weights; histopathology: in all dose groups: individual liver lobules; histopathology of control and high dose groups only: kidneys, spleen, lungs, thymus, stomach, adrenal glands, testes, ovaries; histology of uterus, mammary glands, seminal vesicles, thyroid gland, lungs, pancreas, heart, brain, prostate gland, epididymis, colon, gallbladder, small and large intestines; paired organs weighed separately; end of study: biochemical parameters in serum, blood parameters	Gill et al. 2011
mouse, SPF B6C3F1, 50 ♀, only ♀ animals because of lower spontaneous liver tumour incidence (14%, ♂: 52%) and because both sexes generally react in the same way	**2 years**, 0, 0.5 (n = 100), 1 (n = 75), 2, 4, 8 mg/kg body weight and day, 5 days/week, in corn oil, purity: > 99%, **gavage**	only liver lobes examined histopathologically; **0.5 mg/kg body weight and above**: hepatotoxicity (subcapsular inflammation); **1 mg/kg body weight and above**: statistically significant cytotoxicity in the liver; **4 mg/kg body weight and above**: relative liver weights ↑, foci of changes in hepatocytes, hepatocellular adenomas, hepatocellular adenomas plus carcinomas, number of nodules in hepatocytes ↑; see also section 5.7 **8 mg/kg body weight**: hepatocellular carcinomas, multiplicity of microtumours ↑, latency period up to tumour ↓	Moser et al. 2009

* $p < 0.05$; ** $p < 0.01$

ALT: alanine aminotransferase; ALP: alkaline phosphatase; BrdU: 5-bromo-2′-deoxyuridine; SPF: specific pathogen free; T3: Triiodothyronine; T4: thyroxine

Tab. 3 Findings in the liver of F344 rats 13 weeks after gavage administration of furan (5 days/week) (Gill et al. 2010)

		Dose (mg/kg body weight and day)					
		0	**0.03**	**0.12**	**0.5**	**2**	**8**
biliary tract							
hyperplasia	♂	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	6/12 (50%)	12/12 (100%)
	♀	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	1/12 (8%)	12/12 (100%)
cholangiofibrosis	♂	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	2/12 (17%)	12/12 (100%)
	♀	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	11/12 (92%)
hepatocytes							
apoptosis	♂	3/12 (23%)	4/12 (33%)	6/12 (50%)	12/12 (100%)	12/12 (100%)	12/12 (100%)
	♀	2/12 (17%)	0/12 (0%)	1/12 (8%)	10/12 (83%)	12/12 (100%)	12/12 (100%)
basophilia in cytoplasm	♂	0/12 (0%)	0/12 (0%)	0/12 (0%)	8/12 (67%)	12/12 (100%)	12/12 (100%)
	♀	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	12/12 (100%)	11/12 (92%)
anisokaryosis	♂	0/12 (0%)	0/12 (0%)	0/12 (0%)	0/12 (0%)	10/12 (83%)	12/12 (100%)
	♀	0/12 (0%)	no data	0/12 (0%)	0/12 (0%)	12/12 (100%)	10/12 (83%)
Kupffer cells							
pigmentation	♂	1/12 (8%)	1/12 (8%)	5/12 (42%)	10/12 (83%)	12/12 (100%)	12/12 (100%)
	♀	2/12 (17%)	0/12 (0%)	3/12 (25%)	12/12 (100%)	12/12 (100%)	12/12 (100%)

Tab. 4 Findings in the liver 9 months after oral administration of furan (5 days/week) in male F344 rats (FDA 2015)

	Dose (mg/kg body weight and day)							
	0	**0.02**	**0.044**	**0.092**	**0.2**	**0.44**	**0.92**	**2**
cholangiofibrosis	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	6/20** (30%)	17/20** (85%)	19/20** (95%)
mixed cell foci	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)	0/20 (0%)	1/20 (5%)	5/20* (25%)
biliary duct hyperplasia	1/20 (5%)	1/20 (5%)	0/20 (0%)	1/20 (5%)	0/20 (0%)	0/20 (0%)	6/20* (30%)	19/20** (95%)
subcapsular biliary duct hyperplasia	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)	12/20** (60%)	19/20** (95%)	20/20** (100%)
oval cell hyperplasia	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)	16/20** (80%)
hepatocyte hypertrophy	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)	0/20 (0%)	2/20** (10%)	12/20** (60%)
cytoplasmic changes in the periportal field	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	7/20* (35%)	19/20** (95%)
subcapsular fibrosis	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	3/20 (15%)	14/20** (70%)	20/20** (100%)	20/20** (100%)
subcapsular chronic inflammation	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	2/20 (10%)	14/20** (70%)	20/20** (100%)	20/20** (100%)
subcapsular pigmentation	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	2/20 (10%)	14/20** (70%)	20/20** (100%)	20/20** (100%)

* $p < 0.05$; ** $p \leq 0.01$

Tab. 5 Findings in the liver 15 months after oral administration of furan (5 days/week) in male F344 rats (FDA 2015)

	Dose (mg/kg body weight and day)							
	0	**0.02**	**0.044**	**0.092**	**0.2**	**0.44**	**0.92**	**2**
cholangiofibrosis	0/20 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	10/10** (100%)	10/10** (100%)	8/10** (80%)
mixed cell foci	1/20 (5%)	0/10 (0%)	1/10 (10%)	0/10 (0%)	1/10 (10%)	1/10 (10%)	3/10 (30%)	6/10* (60%)
biliary duct hyperplasia	7/20 (35%)	3/10 (30%)	1/10 (10%)	2/10 (20%)	1/10 (10%)	2/10 (20%)	3/10 (30%)	8/10* (80%)
subcapsular biliary duct hyperplasia	0/20 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	9/10** (90%)	10/10** (100%)	7/10** (70%)
oval cell hyperplasia	0/20 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	3/10* (30%)	7/10** (70%)
hepatocyte hypertrophy	0/20 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	5/10* (50%)	4/10* (40%)	8/10** (80%)
cytoplasmic changes in the periportal field	0/20 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/10 (0%)	1/10 (10%)	7/10** (70%)	8/10** (80%)
subcapsular fibrosis	0/20 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	4/10* (40%)	9/10** (90%)	10/10** (100%)	7/10** (70%)
subcapsular chronic inflammation	0/20 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	8/10** (80%)	9/10** (90%)	10/10** (100%)	7/10** (70%)
subcapsular pigmentation	0/20 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	8/10** (80%)	9/10** (90%)	10/10** (100%)	7/10** (70%)

* $p < 0.05$; ** $p \leq 0.01$

Tab. 6 Findings in the liver 2 months after oral administration of furan (5 days/week) in male F344 rats (FDA 2015)

	Dose (mg/kg body weight and day)							
	0	**0.02**	**0.044**	**0.092**	**0.2**	**0.44**	**0.92**	**2**
cholangiofibrosis	0/149 (0%)	0/150 (0%)	0/99 (0%)	1/100 (1%)	38/50** (76%)	49/49** (100%)	47/50** (94%)	49/49** (80%)
mixed cell foci	7/149 (5%)	7/150 (5%)	6/99 (6%)	3/100 (3%)	5/50 (10%)	3/49 (6%)	6/50 (12%)	13/49** (27%)
basophilic foci	28/149 (19%)	16/150 (11%)	19/99 (19%)	18/100 (18%)	11/50 (22%)	13/49 (27%)	10/50 (20%)	13/49** (27%)
biliary duct hyperplasia	89/149 (60%)	86/150 (57%)	59/99 (60%)	56/100 (56%)	29/50 (58%)	25/49 (51%)	32/50 (64%)	43/49** (88%)
oval cell hyperplasia	14/149 (9%)	15/150 (10%)	10/99 (10%)	8/100 (8%)	7/50 (14%)	6/49 (12%)	14/50** (28%)	33/49** (67%)
regenerative hyperplasia	0/149 (0%)	1/150 (1%)	1/99 (1%)	2/100 (2%)	1/50 (2%)	1/49 (2%)	7/50** (14%)	12/49** (25%)
cytoplasmic vacuolation	23/149 (15%)	23/150 (15%)	14/99 (14%)	19/100 (19%)	12/50 (24%)	18/49** (37%)	23/50** (46%)	37/49** (76%)

* $p < 0.05$; ** $p \leq 0.01$

5.2.3 Dermal application

There are no new data available.

5.3 Local effects on skin and mucous membranes

There are no new data available.

5.4 Allergenic effects

There are no new data available.

5.5 Reproductive and developmental toxicity

There are still no studies specifically of effects on fertility or development.

Oral 90-day studies did not demonstrate effects on the reproductive organs of B6C3F1 mice (Gill et al. 2011) or F344 rats (Gill et al. 2010) up to 8 mg/kg body weight and day (see Table 2).

Another 90-day study with gavage administration reported decreases in absolute and relative prostate weights and seminal vesicle weights in male Wistar rats at 8 mg/kg body weight and day, whereas the weights of the testis and epididymis were not affected. The concentration of luteinizing hormone in serum was reduced, as was the concentration of testosterone at 2 mg/kg body weight and day and above. A dose-dependent increase in apoptosis of Leydig cells, an increase in vacuolation of Sertoli cells, tubular degeneration and germ cell sloughing were described (FDA 2015).

5.6 Genotoxicity

5.6.1 In vitro

Studies of in vitro genotoxicity are shown in Table 7.

5.6.1.1 Furan

Furan did not induce gene mutations in Salmonella typhimurium either with or without the addition of metabolic activation (Mortelmans et al. 1986), unscheduled DNA repair in rat or mouse hepatocytes (Wilson et al. 1992) or DNA strand breaks in mouse lymphoma cells (Kellert et al. 2008).

The results for the induction of sister chromatid exchange were positive in some cases, but they were not regarded as conclusive by the National Toxicology Program (NTP 1993).

In a chromosomal aberration test in CHO (Chinese hamster ovary) cells, aberrations were induced both with and without the addition of metabolic activation at 100 µM and above (NTP 1993); in another chromosomal aberration study, furan was shown to have clastogenic activity only after metabolic activation and the addition of nicotinamide adenine dinucleotide phosphate at 12 500 µg/ml and above (Stich et al. 1981).

In micronucleus tests in human lymphocytes or mouse lymphoma cells, treatment with furan up to cytotoxic concentrations did not induce micronuclei either with or without the addition of metabolic activation (Durling et al. 2007) or without metabolic activation (Kellert et al. 2008).

Furan did not cause changes at the $tk^{+/-}$ locus in mouse lymphoma cells without metabolic activation (Kellert et al. 2008; McGregor et al. 1988; NTP 1993). The evaluation made no distinction between large and small colonies.

5.6.1.2 Metabolite *cis*-2-butene-1,4-dial

In a mutagenicity test with Salmonella typhimurium, *cis*-2-butene-1,4-dial did not induce mutations, with the exception of positive results obtained in the aldehyde-sensitive strain TA104 (Peterson et al. 2000). *cis*-2-Butene-1,4-dial was incubated with Salmonella typhimurium TA104 for 30 minutes, the DNA was isolated and, after preparation,

analysed for DNA adducts by mass spectroscopy. A dose-dependent increase in deoxycytidine and deoxyadenosine adducts was observed (Byrns et al. 2006; Peterson 2006).

In a comet assay in mouse lymphoma cells, DNA strand breaks and DNA cross-links were detected at 25 and 100 µM and above, respectively (Kellert et al. 2008). *cis*-2-Butene-1,4-dial did not induce micronuclei in mouse lymphoma cells up to cytotoxic concentrations, but gene mutations were detected at the $tk^{+/-}$ locus at a relative cell growth of only 30% (Kellert et al. 2008).

In mouse embryonic fibroblasts transgenic for *cII*, the number of mutations was not increased, but the increase in the mutation spectrum to transversions (AT⟶CG) was statistically significant (Terrell et al. 2014).

Tab. 7 Studies of the genotoxicity of furan and the metabolite *cis*-2-butene-1,4-dial in vitro

End point	Test system	Concentration	Effective concentration	Cytotoxicity	Result		Comments	References
					–m. a.	+m. a.		
Furan								
gene mutation (SMT)	TA98 TA100 TA1535 TA1537	0–3333 µg/plate		no data	– – – –	– – – –	S9 mix from hamster and rat livers	Mortelmans et al. 1986
SCE	CHO cells	0–160 µg/ml (–m. a.)	1.6 µg/ml and above	tested up to cytotoxicity (no other details)	+		test 1: +; test 2: (+)	NTP 1993
		0–500 µg/ml (+m. a.)	500 µg/ml			+/–	result not conclusive	
DNA repair synthesis (UDS)	primary rat and mouse hepatocytes	up to 10 mM (680 µg/ml)		no data	–		according to control determinations, adequate exposure in spite of high volatility of furan	Wilson et al. 1992
DNA damage, comet assay	L5178Y mouse lymphoma cells	nominal: 0, 250, 500, 1000, 2000, 4000 µg/ml (determined: 0, 225, 450, 900, 1600, 3100 µM)		–	–	n. t.	concentration in the control medium with UV absorption determined at 211 nm	Kellert et al. 2008
chromosomal aberration	CHO cells	from figure: 0, 20, 40, 50, 100, 120, 184, 220 mM (0, about 1400, 2700, 3400, 6800, 8200, 12 500, 15 000 µg/ml)	184 mM and above (12 500 µg/ml and above)	no data	–	+	S9 mix with NADP: + S9 mix without NADP: –	Stich et al. 1981
chromosomal aberration	CHO cells	0, 100, 500, 1000 µg/ml	100 (–m. a.) or 500 (+m. a.) µg/ml and above	no data	+	+	dose-dependent	NTP 1993
micronucleus (CBMN)	human lymphocytes	1): 0–100 mM (–m. a.) 2): 0–25 mM (+m. a.)		–m. a.: 15 mM +m. a.: 15 mM	–	–	100 or 25 mM: analysis not possible because of 100% cytotoxicity	Durling et al. 2007
micronucleus (CBMN)	L5178Y mouse lymphoma cells	nominal: 0, 250, 500, 1000, 2000, 4000 µg/ml (determined: 0, 225, 450, 900, 1600, 3100 µM)		–	–	n. t.		Kellert et al. 2008

Tab. 7 *(continued)*

End point	Test system	Concentration	Effective concentration	Cytotoxicity	Result		Comments	References
					–m. a.	+m. a.		
gene mutation ($tk^{+/-}$)	L5178Y mouse lymphoma cells	0–2600 µg/ml	1) – 2) 2600 µg/ml 3) 1139 µg/ml	RTG: reduced by 20%–60%	– +/– +/–		increase in mutant frequency in the cytotoxic range (no data for colony count)	McGregor et al. 1988; NTP 1993
gene mutation ($tk^{+/-}$)	L5178Y mouse lymphoma cells	nominal: 0, 250, 500, 1000, 2000, 4000 µg/ml (determined: 0, 225, 450, 900, 1600, 3100 µM)		–	–	n. t.	no data for colony count	Kellert et al. 2008
cis-2-Butene-1,4-dial								
gene mutation (SMT)	TA97 TA98 TA100 TA102 SMT + GSH	0, 1.4, 1.7, 2.1, 2.9, 4.3 µmol/plate (0, about 118, 143, 177, 244, 362 µg/plate)		–	– – – – –	– – – – –	S9 mix from rat livers	Peterson et al. 2000
	TA104 (aldehyde-sensitive strain) TA104 + GSH preincubation	0, 1.4, 1.7, 2.1, 2.9, 4.3 µmol/plate (0, about 118, 143, 177, 244, 362 µg/plate)		244 µg/plate and above	– at non-cytotoxic concentration	n. t.	formation of bis-GSH conjugates; not reproducible (Kellert et al. 2008; no data)	Peterson et al. 2000
DNA adduct	Salmonella TA104	0, 1.42, 2.14, 2.86 mM (0, about 119, 180, 240 µg/ml)	2.14 mM (about 180 µg/ml)		+	n. t.	with cytidine, guanosine, adenosine; not with thymidine	Byrns et al. 2006; Peterson 2006
DNA damage, comet	L5178Y mouse lymphoma cells	0, 6.3, 12.5, 25, 50 µM (0, about 0.53, 1.05, 2.1, 4.2 µg/ml)	25 µM (2.1 µg/ml)	50 µM (4.2 µg/ml)	+ (1.6-fold)	n. t.	cytotoxicity: 50 µM: 50% viable cells, 25% apoptotic cells; plating efficiency: < 30%, RTG: < 10%	Kellert et al. 2008
DNA cross-links, comet		0, 1, 10, 100, 1000 µM (0, about 0.084, 0.84, 8.4, 84 µg/ml)	100 µM (8.4 µg/ml)	100 µM (8.4 µg/ml)	+	n. t.	DNA migration increased after treatment; 100 µM and above: DNA fragmentation of chromatin (apoptotic cells)	Kellert et al. 2008
micronucleus (CBMN)		0, 6.3, 12.5, 25, 50 µM (0, about 0.53, 1.05, 2.1, 4.2 µg/ml)		50 µM (4.2 µg/ml)	–	n. t.	no dose-response relationship cytotoxicity: 50 µM: 50% viable cells, 25% apoptotic cells; plating efficiency: < 30%, RTG: < 10%	Kellert et al. 2008

Tab. 7 *(continued)*

End point	Test system	Concentration	Effective concentration	Cytotoxicity	Result		Comments	References
					–m. a.	+m. a.		
gene mutation ($tk^{+/-}$)		0, 6.3, 12.5, 25, 50 µM (0, about 0.53, 1.05, 2.1, 4.2 µg/ml)	25 µM (2.1 µg/ml)	50 µM (4.2 µg/ml)	+	n. t.	cytotoxicity: 50 µM: 50% viable cells, 25% apoptotic cells; plating efficiency: < 30%, RTG: < 10%	Kellert et al. 2008
cII transgene; mutation spectrum	embryonic Big Blue mouse fibroblasts	0–10 µM; only 30-minute treatment in PBS; 48 hours after treatment, cultivation in large vessels, up to $6–10 \times 10^6$ cells, (72 hours), DNA isolation		dose-dependent ↑, 10 µM: 100% cytotoxicity; decrease in viability; complete culture medium neutralized the toxicity of *cis*-2-butene-1,4-dial	–	n. t.	transversions ↑: AT⟶CG (statistically significant)	Terrell et al. 2014

CBMN: cytokinesis-block micronucleus; GSH: glutathione; m. a.: metabolic activation; NADP: nicotinamide adenine dinucleotide phosphate; n. t.: not tested; PBS: phosphate buffered saline; RTG: relative total growth; SCE: sister chromatid exchange; SMT: Salmonella mutagenicity test; UDS: unscheduled DNA synthesis

5.6.2 In vivo

In vivo genotoxicity data are shown in Table 8. Data are available only for furan, but not for its metabolite *cis*-2-butene-1,4-dial.

5.6.2.1 Drosophila

Furan did not induce sex-linked recessive lethal mutations in the SLRL test in Drosophila (IARC 1995).

5.6.2.2 Mammals

^{14}C-labelled DNA adducts were found in the liver and kidneys of rats given a single gavage dose of furan. The *cis*-2-butene-1,4-dial metabolite did not form adducts with the bases adenine, cytosine or guanine (Neuwirth et al. 2012). In mice, single intraperitoneal and gavage doses of furan of up to 350 and 100 mg/kg body weight, respectively, did not induce either sister chromatid exchange or unscheduled DNA repair (Neuwirth et al. 2012; NTP 1993; Wilson et al. 1992). The comet assay revealed an increase in DNA damage in the liver of mice and rats after a single gavage dose of 250 mg/kg body weight or doses of 16 mg/kg body weight and above (Cordelli et al. 2010; Ding et al. 2012), gavage doses of 2 mg/kg body weight and day for 28 days with a 14-day recovery period (Neuwirth et al. 2012) and gavage doses of 8 mg/kg body weight and day and above for 8 weeks (McDaniel et al. 2012). However, DNA strand breaks were not induced in the liver of mice after 4-week treatment with up to 15 mg/kg body weight and day (Cordelli et al. 2010) or in the spleen after a single or 4-week treatment of mice with gavage doses (Leopardi et al. 2010). In rats, no increase in DNA strand breaks was observed either in the bone marrow after a single treatment (Ding et al. 2012) or in the bone marrow and liver after 5-day and 28-day treatment, respectively (Neuwirth et al. 2012). In mice, DNA damage was detected by γH2AX staining in the spleen (Leopardi et al. 2010) at 8 mg/kg body weight and day and above after 4-week gavage treatment and stimulation of proliferation by concanavalin A; however, no DNA damage was observed in the liver up to furan doses of 15 mg/kg body weight and day (Cordelli et al. 2010). The number of DNA cross-links was not increased in the liver or spleen after a single treatment with doses of up to 15 mg/kg body weight (Cordelli et al. 2010; Leopardi et al. 2010).

The increase in the number of polyploid cells in the liver of mice was statistically significant after treatment with gavage doses of up to 15 mg/kg body weight and day for 4 weeks, whereas there was no change in DNA methylation (Cordelli et al. 2010).

In the bone marrow of mice, chromosomal aberrations were induced concurrently with cytotoxic effects 36 hours after a single intraperitoneal injection of 250 mg/kg body weight, but not 17 hours after the administration of 350 mg/kg body weight (NTP 1993). When rats were given gavage doses for 28 days, the incidence of chromosomal aberrations was increased in splenocytes at 0.5 mg/kg body weight and day and above, whereas in the bone marrow the induction of chromosomal aberrations or micronuclei was not observed up to 2 mg/kg body weight and day (Neuwirth et al. 2012).

In splenocytes that were stimulated for proliferation, micronuclei were not induced in mice after a single or 4-week treatment with gavage doses of up to 250 mg/kg body weight or 15 mg/kg body weight and day (Leopardi et al. 2010) or after a single intraperitoneal or subcutaneous injection of 300 or 275 mg/kg body weight (Durling et al. 2007). Likewise, micronuclei were not detected in the peripheral blood of rats after a single, 1-week or 8-week treatment with gavage doses of up to 16 mg/kg body weight or up to 30 mg/kg body weight and day (Ding et al. 2012; McDaniel et al. 2012).

In Big Blue rats, gene mutations were not observed in the *cII* transgene in the liver, in the *Hprt* gene in the T lymphocytes of the spleen or in the *Pig-a* gene in the peripheral blood up to doses of 30 mg/kg body weight and day (McDaniel et al. 2012). Likewise, the incidence of gene mutations in the *cII* transgene in the liver of Big Blue mice was not increased up to 15 mg/kg body weight and day, but there was a shift in the mutation spectrum. An increased, but not statistically significant incidence of GC to AT transversions was observed and there was a (statistically significant) decrease in GC to AT transitions (Terrell et al. 2014). In 60% of liver tumours induced by furan, there was a shift in the mutation spectrum of the tumours in the oncogene *Hras* from G to T and G to C transversions in codon 117; according to the authors, this was a direct genotoxic mechanism (Reynolds et al. 1987).

Tab. 8 Studies of the genotoxicity of furan in vivo

Test system	Species	Dose	Result	Comments	References
Drosophila					
SLRL	Drosophila, Canton-S wild-type, 10–20 ♂	0, 10 000 mg/l, 3-day feeding in sucrose, a single abdominal injection with 25 000 mg/l	–		NTP 1993
mammals					
^{14}C DNA adducts, liver, kidneys	rat, F344, ♂, number not specified	0, 0.1, 2.0 mg/kg body weight and day, gavage, single	+	3,4-^{14}C-furan dose, analysis by means of LC-MS, examination 2 hours after substance administration to avoid metabolic conversion of the substance to CO_2	Neuwirth et al. 2012
SCE, bone marrow	mouse, B6C3F1, 5 ♂/group	87.5–350 mg/kg body weight or 25–100 mg/kg body weight, intraperitoneal, single	–	examination 23 and 42 hours after intraperitoneal injection	NTP 1993
SCE, bone marrow	rat, F344, 8 ♂/group	0, 0.1, 0.5, 2.0 mg/kg body weight and day, gavage, 5 days/week, 5, 28 days, 0, 2.0 mg/kg body weight and day, 5 days/week, 28 days with 14-day recovery	–	no data for BrdU administration or cytotoxicity	Neuwirth et al. 2012
DNA repair synthesis (UDS), primary hepatocytes: ^{3}H-thymidine labelling	mouse, B6C3F1, 3 ♂/group	0, 10, 50, 100 mg/kg body weight or 0, 10, 50, 200 mg/kg body weight, gavage, single	–	examination 12 hours after treatment examination 2 hours after treatment	Wilson et al. 1992
DNA repair synthesis (UDS), primary hepatocytes: ^{3}H-thymidine labelling	rat, F344, 3 ♂/group	0, 5, 30, 100 mg/kg body weight, gavage, single	–	examination 2 or 12 hours after treatment	
DNA damage, cross-links, liver, (comet assay, pH: 13) standard method	mouse, B6C3F1, 5–6 ♂/group	0, 15, 100, 250 mg/kg body weight, gavage, single	+ 250 mg/kg body weight	examination after 3 hours: effects due to DNA strand breaks, alkali-labile sites or DNA repair intermediates; 100 mg/kg body weight and above: signs of toxicity; histopathology: diffuse change in the parenchyma with areas of necrosis	Cordelli et al. 2010

Tab. 8 *(continued)*

Test system	Species	Dose	Result	Comments	References
DNA damage, spleen, not stimulated, (comet assay, pH: 13)	mouse, B6C3F1, 5 ♂/group	0, 15, 100, 250 mg/kg body weight, gavage, single	–	examination after 3 hours	Leopardi et al. 2010
DNA damage, spleen, not stimulated, (comet assay, pH: 13)	mouse, B6C3F1, 6 ♂/group	0, 2, 4, 8 or 15 mg/kg body weight and day, gavage, 5 days/week, 28 days	–	examination 24 hours after last treatment: no cytotoxicity or toxicity; histopathology of the spleen: changes in the white pulp (T cell region, B cell region); no change in apoptotic cells	
DNA damage, spleen, proliferating, (γH2AX assay)			+ at 8 mg/kg body weight and above	examination 24 hours after last treatment	
DNA cross-links, spleen, (comet assay)		8, 15 mg/kg body weight and day, gavage, 5 days/week, 28 days	–	examination 24 hours after last treatment, no other details	
DNA damage, liver, (comet assay, pH: 13)	mouse, B6C3F1, 6 ♂/group	0, 2, 4, 8 or 15 mg/kg body weight and day, gavage, 5 days/week, 28 days	–	examination 24 hours after last treatment: no signs of toxicity; 4 mg/kg body weight and day and above: slight increase in relative liver weights; 8 mg/kg body weight and day and above: regenerative hyperplasia, BrdU-positive cells ↑; apoptotic cells ↑; genes for cell proliferation, macrophage response, oxidative stress/ROS DNA damage ↑; genes for inflammatory markers (interleukins) ↓	Cordelli et al. 2010
DNA damage, liver, (γH2AX assay)			–		
DNA content, liver, (flow cytometer)			+ at 15 mg/kg body weight and day	small, but statistically significant increase in polyploid cells (8N)	
DNA methylation, liver, (incorporation of ^{3}H-methyl groups by bacterial CpG methylase in genomic DNA)			–		

Tab. 8 *(continued)*

Test system	Species	Dose	Result	Comments	References
DNA damage, liver, (comet assay, pH: 13)	rat, F344, **I**: 6 ♂/group, **II**: 6–7 ♂/group	**I:** 0, 16 mg/kg body weight, **II**: 0, 2, 4, 8, 12, 16 mg/kg body weight, gavage, 4× (0, 24, 48, 69 hours)	**I**: + **II**: +	**I**: examination 1, 3, 6, 8, 16, 24 hours after treatment; DNA tail length: without pre-treatment between 1–8 hours after treatment ↑ (statistically significant); pre-treatment with EndoIII: 3–8 hours after treatment ↑; FPGI: 1–3 hours after treatment ↑ **II**: examination 72 hours after treatment, DNA tail length: 8 mg/kg body weight and above: without pre-treatment ↑ (statistically significant); 4 mg/kg body weight and above: pre-treatment with EndoIII: ↑; 12 mg/kg body weight and above: FPGI: ↑ histopathology: dose-dependent increase: apoptosis, hypertrophy, necrosis, leukocyte infiltration; cell proliferation (Ki-67): dose-dependent increase; statistically significant change in expression of functional genes responsible for apoptosis, cell cycle arrest and DNA repair at 2 mg/kg body weight and above	Ding et al. 2012
DNA damage, bone marrow, (comet assay, pH: 13)		**I**: see above **II**: see above	**I**: – **II**: –	no change in DNA tail length; no influence by pre-treatment with EndoIII or FPGI	
DNA damage, liver, (comet assay, pH: 13)	rat, Big Blue, 6 ♂/group	0, 2, 8, 16, 30 mg/kg body weight and day, gavage, 5 days/week, 8 weeks	+ at 16 mg/kg body weight and day and above	examination 24 hours after last treatment: cytotoxicity presumably also responsible for the positive finding	McDaniel et al. 2012
DNA damage, bone marrow, peripheral blood, (comet assay, pH: 13)	rat, F344, 8 ♂/group	0, 0.1, 0.5, 2.0 mg/kg body weight and day, gavage, 5 days/week, 5, 28 days, 0, 2.0 mg/kg body weight and day, 5 days/week, 28 days with 14-day recovery	–		Neuwirth et al. 2012
DNA damage, liver, (comet assay, pH: 13)			– +	without recovery with recovery at 2 mg/kg body weight and day	

Tab. 8 *(continued)*

Test system	Species	Dose	Result	Comments	References
CA, bone marrow	mouse, B6C3F1, 10 ♂/group	0–350 mg/kg body weight	–	examination 17 hours after intraperitoneal treatment	NTP 1993
		0–250 mg/kg body weight, intraperitoneal, single	+	examination 36 hours after intraperitoneal treatment; cytotoxicity at 250 mg/kg body weight and above	
CA, bone marrow	rat, F344, 3 ♂/group	0, 0.1, 0.5, 2.0 mg/kg body weight and day, 5 days/week, 5, 28 days, 0, 2.0 mg/kg body weight and day, 5 days/week, 28 days with 14-day recovery	–	no data for cytotoxicity	Neuwirth et al. 2012
CA, splenocytes (ex vivo)			+	5 days: CA ↑ at 2 mg/kg body weight and day, 28 days: CA ↑ at 0.5 mg/kg body weight and above 28 days + recovery: CA ↑ at 2 mg/kg body weight, no data for cytotoxicity	
gene mutations, liver, *cII* transgene	mouse, B6C3F1 Big Blue, 5 ♀/group	0, 15 mg/kg body weight, gavage, single, 1×/week, 3 weeks, 5 days/week, 6 weeks (the first two doses only 8 mg/kg body weight and day)	–	examination 24 hours after last treatment: serum ALT levels ↑ (statistically significant after 1×, 3-week and 6-week treatment), histopathology: hepatocellular necrosis with reactive leukocytes and atypical mitoses after 6-week treatment (multipolar in hepatocytes, delayed cell cycle and non-attached condensed chromatin); cell proliferation: cells with positive Ki-67 stains ↑ (statistically significant after 6-week treatment), controls: mortality: 2/5	Terrell et al. 2014
cII transgene; mutation spectrum			+	*cII* mutation spectrum: GC⟶AT transitions ↓ (statistically significant), GC⟶CG transversions ↑ (not statistically significant)	
cII transgene; mutation spectrum			+	*cII* mutation spectrum: GC⟶AT transitions ↓ (by 50%, statistically significant), transversions ↑ (statistically significant), GC⟶TA (doubled)	
gene mutation spectrum	mouse, B6C3F1, liver tumours	60% of furan-induced liver tumours mutated at *Kras* or *Hras* (outside codon 61)	+	*Hras* codon 117: GC⟶CG and GC⟶TA transversions; authors: direct genotoxic effect	Reynolds et al. 1987
	rat, Big Blue, 6 ♂/group	0, 2, 8, 16, 30 mg/kg body weight and day, gavage, 5 days/week, 1 or 8 weeks		examination 24 hours after last treatment	McDaniel et al. 2012

Tab. 8 *(continued)*

Test system	Species	Dose	Result	Comments	References
gene mutations, liver, *cII* transgene		8 weeks	–	30 mg/kg body weight and day: body weights ↓ (at the end by 18%); speckled, strawberry-coloured liver	
gene mutations, blood, *Pig-a* (CD59-deficient)		8 weeks	–	30 mg/kg body weight and day: % RET ↑	
gene mutations, splenic lymphocytes, *Pig-a* assay		8 weeks	–		
gene mutations, splenocytes (T lymphocytes), *Hprt* assay		8 weeks	–		
gene mutations, blood, *Pig-a* (CD59-deficient)		1 week	–	% RET: dose-dependent decrease (not statistically significant)	
micronucleus, reticulocytes, flow cytometer		8 weeks	–	30 mg/kg body weight and day: % RET ↑	
micronucleus, reticulocytes, flow cytometer		1 week	–	% RET: dose-dependent decrease (not statistically significant)	
micronucleus, splenocytes (ex vivo)	mouse, B6C3F1, 4–5 ♂/group	0, 15, 100, 250 mg/kg body weight, gavage, single	–	examination 3 hours after last treatment, no cytotoxicity	Leopardi et al. 2010
micronucleus, splenocytes (ex vivo)	mouse, B6C3F1, 6 ♂/group	0, 2, 4, 8 or 15 mg/kg body weight and day, gavage, 5 days/week, 28 days	+ 4 mg/kg body weight and above	examination 24 hours after last treatment, no cytotoxicity; no signs of toxicity; histopathology of the spleen: changes in the white pulp (T cell region, B cell region); no change in apoptotic cells	
micronucleus, bone marrow, comet assay	rat, F344, 8 ♂/group	0, 0.1, 0.5, 2.0 mg/kg body weight and day, gavage, 5 days/week, 5, 28 days, 0, 2.0 mg/kg body weight and day, 5 days/week, 28 days with 14-day recovery	–	no data for cytotoxicity	Neuwirth et al. 2012

Tab. 8 *(continued)*

Test system	Species	Dose	Result	Comments	References
micronucleus, reticulocytes (peripheral blood), flow cytometer	mouse, Balb/C, **I**: 2–3 ♂/group, **II**: 5–6 ♂/group, CBA, **III**: 4 ♂/group	**I**: 0, 50, 75, 90, 110, 125, 150, 175, 200, 250, 300 mg/kg body weight, intraperitoneal, single	–	examination 42 hours after last treatment, no evidence of dose-response relationship; **I**: 300 or 275 mg/kg body weight: signs of reduced physical activity; **II**: 275 mg/kg body weight: 2 animals died	Durling et al. 2007
		II: 0, 150 or 275 mg/kg body weight, subcutaneous, single	–		
		III: 0, 225 mg/kg body weight, intraperitoneal, single	–		
micronucleus, reticulocytes (peripheral blood), flow cytometer	rat, F344, 6–7 ♂/group	0, 2, 4, 8, 12, 16 mg/kg body weight, gavage, 4× (0, 24, 48, 69 hours)	–	examination 3 hours after last treatment, 12 mg/kg body weight and above: % RET: statistically significant decrease histopathology: dose-dependent increase: apoptosis, hypertrophy, necrosis, leukocyte infiltration; cell proliferation (Ki-67): dose-dependent increase; statistically significant change in expression of functional genes of apoptosis, cell cycle arrest and DNA repair at 2 mg/kg body weight and above	Ding et al. 2012

ALT: alanine aminotransferase; BrdU: 5-bromo-2′-deoxyuridine; CA: chromosomal aberration; EndoIII: endonuclease III; FPGI: formamidopyrimidine-DNA glycosylase; RET: reticulocytes; SCE: sister chromatid exchange; SLRL: sex-linked recessive lethal

5.6.3 Summary

In vitro, furan has clastogenic potential and the metabolite *cis*-2-butene-1,4-dial is mutagenic in the aldehyde-sensitive Salmonella strain TA104. In addition, *cis*-2-butene-1,4-dial forms adducts in vitro with deoxyribonucleotides of guanine, cytosine and adenine. Likewise, mutations that were not observed in spontaneous liver tumours were found in the activated oncogenes of liver tumours induced by furan (Hartwig and MAK Commission 2018).

The following conclusions are drawn on the basis of the new data:

The low boiling point, the selection of the cell line, the absence of suitable activation enzymes (CYP2E1 is not present in L5178Y cells and has only low activity in routinely induced rat liver S9) or the presence of detoxification systems all contribute to the inconsistency of the in vitro genotoxicity test results that were obtained with furan. The reactive furan metabolite, *cis*-2-butene-1,4-dial, is mutagenic in vitro. However, clear evidence of an association between the metabolism of furan and genotoxicity in the cell is difficult to provide because of the high chemical reactivity of *cis*-2-butene-1,4-dial. Furan is activated extracellularly in many test systems. As *cis*-2-butene-1,4-dial reacts rapidly with the protein nucleophiles, it probably does not reach the nuclear DNA.

Most in vivo genotoxicity tests with furan yielded negative results. Tests in Big Blue rats and mice revealed neither increases in DNA damage in the bone marrow and spleen or micronuclei in the blood and spleen, nor mutations in the *Hprt* gene in the spleen, in the *Pig-a* gene in the blood or spleen and in the *cII* transgene in the liver. The comet

assay revealed DNA damage in the liver as the target organ in rats and mice and a shift in the mutation spectrum from GC to TA transversions in mice.

The extent of furan metabolism in vitro or in vivo is not known. It is possible that such a small amount of furan is metabolized that the test systems (even the most sensitive methods) are not sensitive enough to detect a genotoxic mechanism (Durling et al. 2007).

Only in vitro data are available for the metabolite of furan, *cis*-2-butene-1,4-dial. The data show that for the narrow concentration range in which the genotoxicity of the substance can be investigated, the contribution of genotoxic effects of *cis*-2-butene-1,4-dial to the development of the tumours induced by furan cannot be completely excluded. Concentrations that double the control values in genotoxicity tests are associated with a decrease in plating efficiency, relative growth and survival. The cytotoxicity of *cis*-2-butene-1,4-dial seems to be the predominant factor for the toxicity and carcinogenicity of furan (Kellert et al. 2008).

Furan induces GC to TA transversions in the *cII* transgene in the liver and is genotoxic via secondary mechanisms at cytotoxic concentrations that are accompanied by inflammation and cell proliferation.

5.7 Carcinogenicity

As described in the 2006 documentation (Hartwig and MAK Commission 2018), the NTP carcinogenicity study in F344 rats yielded cholangiocarcinomas (86%–98%) at the lowest dose tested of 2 mg/kg body weight and day and above; in addition, hepatocellular adenomas or carcinomas, mononuclear leukaemia and damage to the haematopoietic system were observed at higher doses. Likewise, hepatocellular adenomas or carcinomas and phaeochromocytomas in the adrenal medulla were found in mice at the lowest dose tested of 8 mg/kg body weight and day and above.

New studies have since been carried out (see Table 9).

An oral two-year study with female B6C3F1 mice used more animals in the low dose groups for a more precise determination of the onset of hepatotoxicity; histopathological examination was carried out only in the liver. Dose-dependent hepatotoxicity was observed at 0.5 mg/kg body weight and day and above. Statistically significant increases in tumour incidences were observed for hepatocellular adenomas or carcinomas at 4 mg/kg body weight and day and above and for hepatocellular carcinomas at 8 mg/kg body weight and day (Moser et al. 2009).

As the two-year gavage study carried out by the NTP (1993) reported that female and male F344 rats reacted with similar sensitivity, only male animals were used in a new two-year study with gavage doses of 0.02 to 2 mg/kg body weight and day. Malignant mesotheliomas were observed in the epididymis or testis with an incidence that narrowly reached statistical significance only at 2 mg/kg body weight and day. The incidence of mononuclear leukaemia was increased at 0.2 mg/kg body weight and day and above; however, as the increased incidence was in the range of the mean and standard deviation of the historical controls of this laboratory, it is not considered to have been caused by the treatment with furan. A higher number of animals was used in the low dose groups (150 or 100 per dose) to increase the likelihood of detecting changes at low dose levels. The objective of the study was to determine the dose–response relationship of cholangiocarcinomas. Cholangiocarcinomas were identified according to the criteria established by Thoolen et al. (2010). On the basis of these criteria, cholangiocarcinomas were not observed in male F344 rats. A follow-up examination of 23 livers from the 2 mg/kg group of the NTP study from 1993 found that only 3 livers had cholangiocarcinomas as defined by the criteria developed by Thoolen et al. (2010) (NTP (1993): 43/50). A follow-up examination of 6 livers from the 8 mg/kg group of the NTP study from 1993 found that all animals had cholangiocarcinomas as defined by the criteria developed by Thoolen et al. (2010) (FDA 2015). Meanwhile the study by FDA (2015) has been published (Von Tungeln et al. 2017).

Tab. 9 Carcinogenicity studies of furan

Author:	Moser et al. 2009						
Substance:	Furan (purity: 99%)						
Species:	**mouse**, B6C3F1, 50 ♀ per group						
Administration route:	oral						
Dose:	0, 0.5 (n = 100), 1 (n = 75), 2, 4, 8 mg/kg body weight and day						
Duration:	2 years, 5 days/week						
Toxicity:	0.5 mg/kg body weight and day and above: dose-dependent hepatotoxicity						
		dose (mg/kg body weight and day)					
		0	**0.5**	**1**	**2**	**4**	**8**
surviving animals	♀	33/50 (66%)	69/100 (69%)	45/75 (60%)	40/50 (81%)	32/50 (64%)	27/50 (54%)
tumours and pre-neoplasms in the liver							
nodules	♀	2/25 (8%)	17–20% (no other data)	17–20% (no other data)	17–20% (no other data)	15/25 (60%)*	22/22 (100%)*
foci of altered hepatocytes terminal rate	♀	3/25 (12%)	5/55 (9%)	4/36 (11%)	5/31 (16%)	10/25 (40%)*	13/22 (59%)*
hepatocellular adenomas terminal rate	♀	3/25 (12%)	3/55 (5%)	4/36 (11%)	3/31 (10%)	8/25 (32%)*	18/22 (82%)*
hepatocellular carcinomas terminal rate	♀	0/25 (0%)	2/55 (4%)	1/36 (3%)	1/31 (3%)	1/25 (4%)	6/22 (27%)*
hepatocellular adenomas or carcinomas terminal rate	♀	3/25 (12%)	5/55 (9%)	5/36 (14%)	4/31 (13%)	9/25 (36%)*	14/22 (64%)*

* $p < 0.05$

Author:	FDA 2015							
Substance:	Furan (purity: 99.5%)							
Species:	**rat**, F344, 50 ♂/group unless otherwise specified							
Administration route:	gavage							
Dose:	0 (n = 190), 0.02 (n = 180), 0.044 (n = 130), 0.092 (n = 130), 0.2, 0.44, 0.92, 2 mg/kg body weight and day for the detection of slight effects at a low dose, interim examination after 9 (n = 20) and 15 (n = 10) months without neoplastic findings							
Duration:	2 years, 5 days/week							
Toxicity:	0.2 mg/kg body weight and above: dose-dependent hepatotoxicity							
	dose (mg/kg body weight and day)							
	0	**0.02**	**0.044**	**0.092**	**0.2**	**0.44**	**0.92**	**2**
surviving animals	45/150 (30%)	42/150 (28%)	28/100 (28%)	28/100 (28%)	16/50 (32%)	13/50 (26%)	12/50 (24%)	08/50 (16%)

Tab. 9 *(continued)*

tumours								
malignant mesotheliomas [a)]								
epididymis	6/149 (4%)	8/149 (5%)	1/98 (1%)	2/100 (2%)	0/50 (0%)	2/50 (4%)	2/50 (4%)	5/50 (10%)
testis	5/150 (3%)	7/149 (5%)	1/98 (1%)	1/100 (1%)	0/50 (0%)	2/49 (4%)	2/50 (4%)	4/50 (8%)
epididymis or testis	6/150 (4%)	8/150 (5%)	1/98 (1%)	2/100 (2%)	0/50 (0%)	2/50 (4%)	2/50 (4%)	6/50 (12%)*
all organs	6/150 (4%)	9/150 (6%)	1/100 (1%)	2/100 (2%)	0/50 (0%)	3/50[b)] (6%)	2/50 (4%)	6/50 (12%)*
mononuclear leukaemia [c)]								
overall rate	47/150 (31%)	56/150 (37%)	36/100 (36%)	44/100 (44%)*	29/50 (58%)**	18/50 (36%)	27/50 (54%)**	28/50 (56%)**

* $p < 0.05$; ** $p < 0.01$ (poly-3 test);
[a)] historical control animals in this laboratory from 1988–2014: 31/742 (4.2%; 0.0%–6.4%);
[b)] with mesothelioma of the atrium of the heart;
[c)] historical control animals in this laboratory from 1988–2014: 348/742 (46.9%; 31.3%–64.6%)

6 Manifesto (MAK value/classification)

There are no inhalation studies available. The critical effect of furan after oral exposure is toxicity, primarily in the liver of rats and mice and in the bile ducts of rats, which leads to carcinomas after long-term exposure.

Carcinogenicity. On the basis of the studies described in the 2006 documentation (Hartwig and MAK Commission 2018) and the studies that have been published since then, it can be concluded that furan is slightly genotoxic only at toxic doses; genotoxicity is therefore regarded as a secondary effect. Studies are now available in the low dose range, which yielded negative results. The 13-week gavage studies in rats (Gill et al. 2010) and mice (Gill et al. 2011) showed that hepatocytes are damaged at lower doses than bile-duct cells (see Section 5.2.2). Two-year gavage studies with male F344 rats (FDA 2015; Von Tungeln et al. 2017) and female B6C3F1 mice (Moser et al. 2009) identified doses at which carcinogenicity was not induced. A NOAEL of 0.092 mg/kg body weight and day was obtained for furan in rats; subcapsular inflammation was found in the liver of mice at the lowest dose tested of 0.5 mg/kg body weight. In the 13-week study, the NOAEL for mice was 0.12 mg/kg body weight and day. As NOAELs are available for mice and rats and a non-genotoxic mechanism of action is considered to be the cause of carcinogenicity, furan has been re-classified in Carcinogen Category 4 and a MAK value can be established.

Germ cell mutagenicity. In vitro studies found that furan has clastogenic potential. The metabolite *cis*-2-butene-1,4-dial is mutagenic and clastogenic in vitro, but it is rapidly inactivated in vivo. In vivo furan induces GC to TA transversions in the *cII* transgene in the liver and is genotoxic via secondary mechanisms only at cytotoxic doses that are accompanied by inflammation and cell proliferation. There are no studies available in germ cells. Toxicity studies revealed lesions of the ovaries and testes only when the animals were exposed to high doses and had already incurred severe liver damage. Therefore, classification in one of the germ cell mutagen categories is not required.

MAK value. Low gavage doses of furan induced hepatotoxicity in rats and mice. In F344 rats, inflammation and apoptosis of hepatocytes were observed in the males at 0.12 mg/kg body weight and day and above after 13 weeks (Gill et al. 2010), and cholangiofibrosis was found at 0.2 mg/kg body weight and day and above after 2 years (FDA 2015), which was detected only at 2 mg/kg body weight and day and above after 13 weeks. The finding that, at 0.44 mg/kg body weight and day, effects were observed in 30% of the animals after 9 months and in 100% of the animals after 15 months indicates that the effects increase with the increasing duration of exposure. A NOAEL of 0.092 mg/kg body weight and day was established.

The inflammation and apoptosis of hepatocytes observed in F344 rats in the 13-week study were not found in F344 rats in the two-year study. This may have been caused by genetic differences in F344 strains from different breeders. Another possible explanation is that these are transient effects that were compensated by the liver after long-term exposure to furan. However, this is not substantiated by the findings from the two-year study, which did not observe apoptosis at the interim examination after 9 months (Beland 2016; FDA 2015). Therefore, inflammation and apoptosis of the hepatocytes of F344 rats after 13-week administration of furan are used as the basis for the derivation of the MAK value. The NOAEL for this effect was 0.03 mg/kg body weight and day.

The following toxicokinetic data are used to extrapolate this NOAEL to a concentration in workplace air: the corresponding species-specific correction value for the rat determined on the basis of the toxicokinetic data (1:4), the established oral absorption (100%), the body weight (70 kg) and the respiratory volume (10 m^3) of the person, and the established 90% absorption by inhalation. This results in a concentration in air of 0.06 mg/m^3 or 0.021 ml/m^3. An intensification of the effects over time is not taken into account because the effects were not observed in the chronic study.

A PBPK model shows that after 4-hour exposure to 10 ml/m^3, exposure to the toxic metabolite *cis*-2-butene-1,4-dial is 3 and 10 times lower in humans than in rats and mice, respectively. The reaction rate is determined by the transport of furan to the liver rather than its metabolism to form the dialdehyde (Kedderis and Held 1996). Therefore, interindividual differences in the expression of the enzyme CYP2E1 in humans have no effect on the concentration of *cis*-2-butene-1,4-dial in the liver. According to these calculations, at the same exposure concentration, the body burden of furan or its metabolite is lower in humans than in rats or mice. Thus, the margin that is generally required between the NOAEL from animal studies and the MAK value is not applicable, and a MAK value for furan of 0.02 ml/m^3 has been established.

This low value is far below the MAK value of 2 ml/m^3 that was established for the irritant hydrogen chloride. Thus, in spite of a lack of data, irritation is not assumed to occur after exposure to furan at the MAK value.

As the MAK value was calculated from an oral study and it was found that, unlike after oral administration, inhalation exposure resulted in lower concentrations in the liver than in the blood (Churchwell et al. 2015), the MAK value provides additional safety.

Peak limitation. Furan has been classified in Peak Limitation Category II because the MAK value was derived from a systemic effect. Furan is eliminated rapidly (85% within 24 hours; Hartwig and MAK Commission 2018); however, the half-life of the reactive metabolite *cis*-2-butene-1,4-dial is not known. Therefore, the default excursion factor of 2 has been established.

Prenatal toxicity. There are no studies of effects on development. Therefore, furan has been classified in Pregnancy Risk Group D.

Absorption through the skin. Studies of the dermal absorption of furan are not available. About 0.5 mg is absorbed after exposure at the MAK value at a respiratory volume of 10 m^3 and 90% absorption by inhalation. On the basis of model calculations, at least 126 mg is absorbed under the standard conditions of exposure (2000 cm^2 surface area of skin exposed to a saturated aqueous solution for 1 hour). Therefore, dermal absorption may significantly contribute to the toxicity of furan, and the substance remained being designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. As clinical findings or results from experimental animal studies are not available for sensitization, furan has not been designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

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